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# Chromatin organization and dynamics in double-strand break repair

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Chromatin is organized and segmented into a landscape of domains that serve multiple purposes. In contrast to transcription, which is controlled by defined sequences at distinct sites, DNA damage can occur anywhere. Repair accordingly must occur everywhere, yet it is inevitably affected by its chromatin environment. In this review, we summarize recent work investigating how changes in chromatin organization facilitate and/or guide DNA double-strand break repair. In addition, we examine new live cell studies on the dynamics of chromatin and the mechanisms that regulate its movement.

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## Chromatin on the move

It has been almost 20 years since John Sedat's laboratory showed that chromatin is mobile using live cell imaging of GFP-tagged loci [1]. At the time this stood in contradiction to datasets from fluorescence recovery after photobleaching (FRAP) [2], and from imaging UV-induced damage within interphase chromosomes [3], which both argued that chromatin position is static. On the other hand, it was obvious that chromatin must be able to move to enable biological events like meiotic homolog pairing, homologous recombination (HR), chromatin condensation and gene activation through long-range enhancer–promoter interactions. The Sedat laboratory resolved this issue by showing that chromatin does indeed move randomly in both *S. cerevisiae* and *D. melanogaster* within constrained volumes, which are on a scale below the resolution of FRAP. This seminal work additionally showed that size does not matter (i.e. a yeast CEN-containing plasmid, which clusters with other centromeres, was no more mobile than a whole chromosome), and that microtubules constrain chromatin movement, at least in yeast [1]. Recent articles now address many of the questions raised by these early studies, the foremost being, 'Does chromatin movement have a biological function and how is it regulated?'

## DNA damage induces chromatin mobility

Double-strand break (DSB) repair by homologous recombination with an ectopic or non-sister donor sequence requires a physical search for the homologous template. This has long been considered one of the central mechanisms that would require chromatin movement. Investigations into this hypothesis led to the discovery that endonuclease-induced DSBs in budding yeast move more than uncleaved loci [4,5]. This is regulated by the DNA damage checkpoint kinase, Mec1-Ddc2 (or ATR-ATRIP in mammals). Intriguingly, an induced DSB affects more than just the surrounding chromatin: the Rothstein group was first to report an apparent increase in chromatin mobility for genomic loci far from the break site [5]. Later work confirmed this generalized increase in chromatin mobility [6], which, although less pronounced than DSB movement, was ATP-dependent, sensitive to the number of DSBs induced, and dependent on checkpoint kinase activation, including the downstream kinase, Rad53 [6]. This link was recently shown relevant for mammalian cells, as ionizing radiation (IR)-induced damage triggers increased locus movement, in a manner dependent on the repair factor 53BP1 and the ATM kinase, 53BP1 [7••].

A recent study by the Durocher group proposed an essential budding yeast kinetochore protein, Cep3, as the relevant target of the checkpoint kinase that controls chromatin movement, both locally and globally [8••]. The authors suggested that a point mutation, *cep3-S575A*, which compromises a Rad53 phosphoacceptor site in Cep3, completely abrogated the enhanced movement that accompanies a targeted DSB, as well as the global chromatin movement response [4]. The authors hypothesized that damage-induced phosphorylation of Cep3 triggers a release of centromeres from the interphase spindle that links them to a membrane spanning spindle pole body (SPB). This release is proposed to generally enhance chromatin movement. They did not detect any change in distance between the SPB and yeast centromeres

following cut induction, but they did score, using a relative mean square displacement assay, an enhanced relative mobility between the SPB and a centromere, which was dependent on the phosphoacceptor site in Cep3. Linking this to damage, they showed that cells treated with Zeocin, a radiomimetic drug previously used to induce global chromatin mobility [6], led to the declustering of kinetochores near the SPB. However, the *cep3-S575A* mutation had no effect on repair by homologous recombination. Unfortunately, the study failed to monitor the efficiency of DSB induction in the *cep3-S575A* mutant, leaving alternative interpretations possible for the lack of increased mobility (i.e. less efficient cleavage or impaired checkpoint activation, would similarly fail to increase mobility). Given that there are significant differences in basal level mobility between G1- and S-phase chromatin [9], cell cycle effects must also be carefully controlled for. Nonetheless, this study raises the question whether enhanced movement is really necessary for homology search.

Clearly, not all damage in yeast triggers enhanced movement [9], nor does all damage activate the Mec1-Ddc2/Rad53 checkpoint. Spontaneous damage or DNA-protein adducts that are repaired by exchange with a sister chromatid, or by precise non-homologous end-joining, appear not to trigger changes in chromatin mobility [9], nor do they shift to the nuclear periphery for repair [10]. Too much movement at a DSB was, moreover, deleterious, particularly in repetitive regions in mammalian cells where extensive movement correlated with translocations and deletion events [11]. Intriguingly, the rate of misrepair was strongly affected by the position of the observed locus in the nucleus in both yeast and mammals [12,13,14,15]. This initiated an examination of how nuclear compartments, which often stem from local chromatin structure [16], influence pathways of repair. It was observed that breaks in heterochromatin behaved differently from breaks in euchromatic zones particularly in mammals and flies [17,18,19,20••]. Thus, chromatin movement can provide a means to escape an unfavorable chromatin compartment or access a set of factors that were unavailable in the lesion's original context. Telomeres are an excellent case in point: they are highly repetitive, yet when unprotected, they act like a single-ended DSB [21]. This raised the question whether the mobility of telomeres is controlled and whether their movement affects telomere maintenance during end uncapping.

A study by the Greenberg laboratory investigated what happens to telomere movement during repair or maintenance by recombination-dependent pathway called alternative lengthening of telomeres, or ALT. They found that DSB signaling at an ALT telomere causes long range movement and clustering of chromosome ends, which is thought to favor homology-driven maintenance of telomere repeats [22••]. The alternative, i.e. activating a DSB response at a telomere, can be dangerous. Previous work from the de Lange laboratory had shown that uncapped telomeres (which lack the protective telomere binding protein TRF2) show increased movement, which correlated with enhanced rates of telomere end-to-end fusion. Both movement and end-to-end fusion depended on 53BP1 [23]. Recent work from this group investigated telomere damage further and showed that SUN-domain-containing proteins, which bridge from the nucleoskeleton to the cytoskeleton in the LINC complex (see below), promote increased dynamics of dysfunctional uncapped telomeres, enhancing the rate of untimely end-to-end fusions by NHEJ [7••]. The authors also showed a role for cytoskeleton-bound kinesins in telomere fusions and the repair of internal breaks, suggesting that an active, kinesin-driven movement of the nucleus or elements in the nuclear envelope affect DSB repair. This is reminiscent of a study in yeast which showed that kinesins can promote movement of subtelomeric DSBs [24•]. In summary, increased movement of a telomere can be useful for ALT-like telomere recombination, yet is deleterious in conditions that generate uncapped or dysfunctional ends, for it leads to telomere-telomere fusions. The next section will discuss new articles that look at the effect of chromatin structure, actin and microtubules on chromatin motion.

## Chromatin structure, actin and microtubules affect chromatin mobility

The budding yeast genome is organized in a Rabl configuration where the centromeres are attached to the SPB and the telomeres are attached to the periphery [25,26]. Forced detachment of the centromere from the SPB increases chromatin movement [8••,27], as does telomere release from the periphery [28] or the loss of anchorage by ablation of SIR-mediated silencing [8••,29]. However, a chromosome that is detached from its perinuclear anchor is still more confined than a free-floating plasmid ring [8••,30], suggesting that there are additional constraints on chromosomes. One constraint stems from the inherent structure of the chromatin fiber, while the second is the tethering of sister chromatids through cohesin [9]. Consistently, there is accumulating evidence that supports the notion that altered chromatin fiber organization, that is, nucleosome eviction or remodeling, increases movement. Notably, the targeting of a functional nucleosome remodeler, INO80, to a chromosomal locus [30,31••] or the INO80-dependent eviction of nucleosomes at the *PHO5* locus in the absence of phosphate, both increase the movement of an appropriately tagged locus.

Interestingly, DNA damage also changes chromatin structure. A new study in yeast shows that Zeocin-induced damage leads to the degradation of ~30% of the four core histones within a short time [32]. This induces chromatin decompaction, and increases both the flexibility of the chromatin fiber and its mobility, in manner dependent on the DNA damage checkpoint and INO80. Furthermore,

either the artificial reduction of histone proteins H3/H4, or the use of a mutant that naturally has lower levels of histones (*nhp6Δ*), triggers decompaction and increased chromatin movement [32]. This result contradicts an earlier report where the shutdown of histone H3 production was proposed to decrease locus mobility [27]. The difference may reflect that fact nucleosome depletion and enhanced chromatin flexibility requires the loss of both H3 and H4. Furthermore, it was shown that H4 (but not H3) shutdown leads to a declustering of kinetochores [33], an event that may also contribute to the increased chromatin movement observed by Hauer *et al*. The influence of inherent chromatin structure on mobility is consistent with the finding that histone modifications correlate with the propensity for translocations in mammalian cells [16]. A very recent paper [34••] also documents a similar unfolding and expansion of chromatin in response to UV-induced damage in mammalian cells, although in this case the effect stem largely from histone mobilization and replacement, rather than degradation [34••].

Besides inherent changes in chromatin structure, accumulating evidence also implicates microtubules and the actin cytoskeleton as drivers of nuclear and/or chromatin movement. In Sedat's study, the depolymerisation of microtubules by Nocodazole was shown to increase chromatin movement in budding yeast [1]. This suggested that microtubules mediated constraint, although it was not clear whether this effect arose from direct interactions between chromatin and microtubules or indirect contact through the nuclear envelope. The LINC complex can connect cytoskeletal filaments [35] through Klarsicht, ANC-1, and Syne homology proteins (KASH also known as Nesprin) on the outer nuclear membrane, to their ligands, the SUN-domain proteins, which span the perinuclear space and protrude into the nucleoplasm. Some SUN-domain proteins interact with chromatin, specifically telomeres [35], and resected DSBs in budding yeast [36,37,38••,39•].

Work from the de Lange laboratory showed for the first time that, in contrast to yeast, the treatment of mammalian cells with dysfunctional telomeres with the microtubule poisons Taxol or Nocodazole actually decreased their movement in a reversible manner [7••]. Importantly, the authors showed that removal of SUN1/2, an essential bridge from the cytoskeleton to the inner nuclear membrane, decreased movement, similar to the microtubule poisons. The reduced movement, due either to depolymerization of the cytoskeleton or loss of this cytoskeleton-to-nucleus link, also reduced the rate of telomere-telomere fusions. Importantly, Taxol treatment also seemed to decrease the movement of IR-induced foci, and not only dysfunctional telomeres. This implies that the forces applied to the chromosomes through the microtubules can be transduced to internal chromatin. Whereas a mechanism through which cytoskeleton-associated kinesins drive SUN-domain-bound telomeres into a clustered, bouquet arrangement is well-characterised in meiotic prophase, this checkpoint kinase-induced event in mitosis does not entail bouquet formation and is most likely differently regulated.

In budding yeast, as mentioned above, the depolymerization of microtubules had the opposite effect on chromatin movement: mobility increased after Nocodazole treatment, consistent with data showing that the deletion of *CSM4*, a putative LINC protein, similarly led to increased subtelomere movement [31••]. This may be due to the loss of microtubules that tether interphase centromeres to the SPB [40], or the disruption of a network of intranuclear microtubules [41], something quite unique to budding yeast. It is noteworthy that in meiosis, bouquet formation is also driven by cytoplasmic actin filaments in budding yeast, rather than microtubules, suggesting that in this species actin filaments replace microtubules for some aspects of nuclear movement.

Nonetheless, in all eukaryotes, actin forms a cytoplasmic network of filaments and it is found, at least in its monomeric 'G' form, inside the nucleus in a range of protein complexes, the most prominent of which are chromatin remodelers [42]. Work from the Fabre laboratory has recently shown that both cytoplasmic and nuclear actin contribute to chromatin motion, through a mechanism that appears to be independent of the putative budding yeast LINC [31••]. Treatment of yeast cells with the actin filament poison Latrunculin A (LatA) was sufficient to decrease the movement of a locus. While this suggests that cytoplasmic actin filaments might move the yeast nucleus, much like microtubules do in *S. pombe* and mice, it is also possible that LatA affects movement indirectly by altering nuclear G-actin. Intriguingly, the targeting of the actin-containing remodeler INO80, which increases the movement of a locus under normal conditions [30], fails to do so when cells are treated with LatA. This result suggests that LatA may bind nuclear actin and disrupt the function of the INO80 complex [42]. This mechanism might affect other actin-containing chromatin modulating complexes, as well, such as NuA4 (TIP60), Swi/Snf, or SWR1 (SRCAP). Since INO80 is necessary for the eviction and degradation of histones in response to DNA damage [32], LatA could interfere with INO80-mediated changes in the nucleosome packing, thereby abrogating the damage-associated increase in chromatin mobility.

## The effects of subnuclear chromatin organization on DNA repair

There is no doubt that chromatin movement exists, and is enhanced by some types of DNA damage; but the question persists, why? It has been proposed that chromatin compartments affect the efficiency of certain repair pathways,

or, at the very least, favor/disfavor certain damage processing steps. This last section will examine how subnuclear compartments, like the nuclear envelope and the nucleolus, affect DSB repair.

The nuclear pore complex (NPC) contains > 30 different nuclear pore proteins (nucleoporins), creating a complex with eight-fold symmetry that spans the nuclear envelope and gates traffic between the cytoplasm and the nucleus [43]. In budding yeast, the NPC is a binding site for persistent DSBs [10,36,37] including breaks that occur at collapsed forks [10,44•] or in subtelomeric regions [45]. In addition, embedded in the inner nuclear membrane is the Sad1-Unc-84-related (SUN) domain protein Mps3, which acts as an alternative binding site for resected DSBs in S phase [37]. This same phenomenon occurs in fission yeast [39•]. DSB break recruitment to either the NPC or to Mps3/Sad1 has different requirements than recruitment to pores [37,38••], and appears to favor distinct features of repair.

DSB recruitment to the NPC is independent of cell-cycle stage, does not require the recombinase Rad51 nor the INO80 chromatin remodeling complex, and is independent of extensive resection (at least in G1 phase cells; [8••,30,38••]). In contrast, Mps3-DSB interaction occurs in S/G2 phase, requires resection, the ssDNA binding factor, Rad51, and INO80. Importantly, the SWR1 chromatin remodeler and its deposition of Htz1 (H2A.Z) at breaks, contributes to the peripheral relocation to either site of anchorage. The outcomes of relocation are deduced from the phenotypes that arise from ablation of one or the other anchors. Based on such an analysis, it would seem that that Mps3 helps suppress illegitimate recombination, perhaps by anchoring or protecting the resected ends until an appropriate template appears [39•]. The NPC complex, on the other hand, appears to promote alternative repair pathways, such as template switching at a broken replication fork, or BIR at single-ended breaks [46••]. The Durocher group finds that Cohibin (a complex consisting of Lsr4-Csm1 and kinesin-14) is necessary for a subtelomeric DSB and the NPC to interact [24•]. Lsr4-Csm1 is involved in rDNA stabilization through perinuclear anchoring [47], but it has not been implicated the recovery from persistent DSBs or collapsed replication forks.

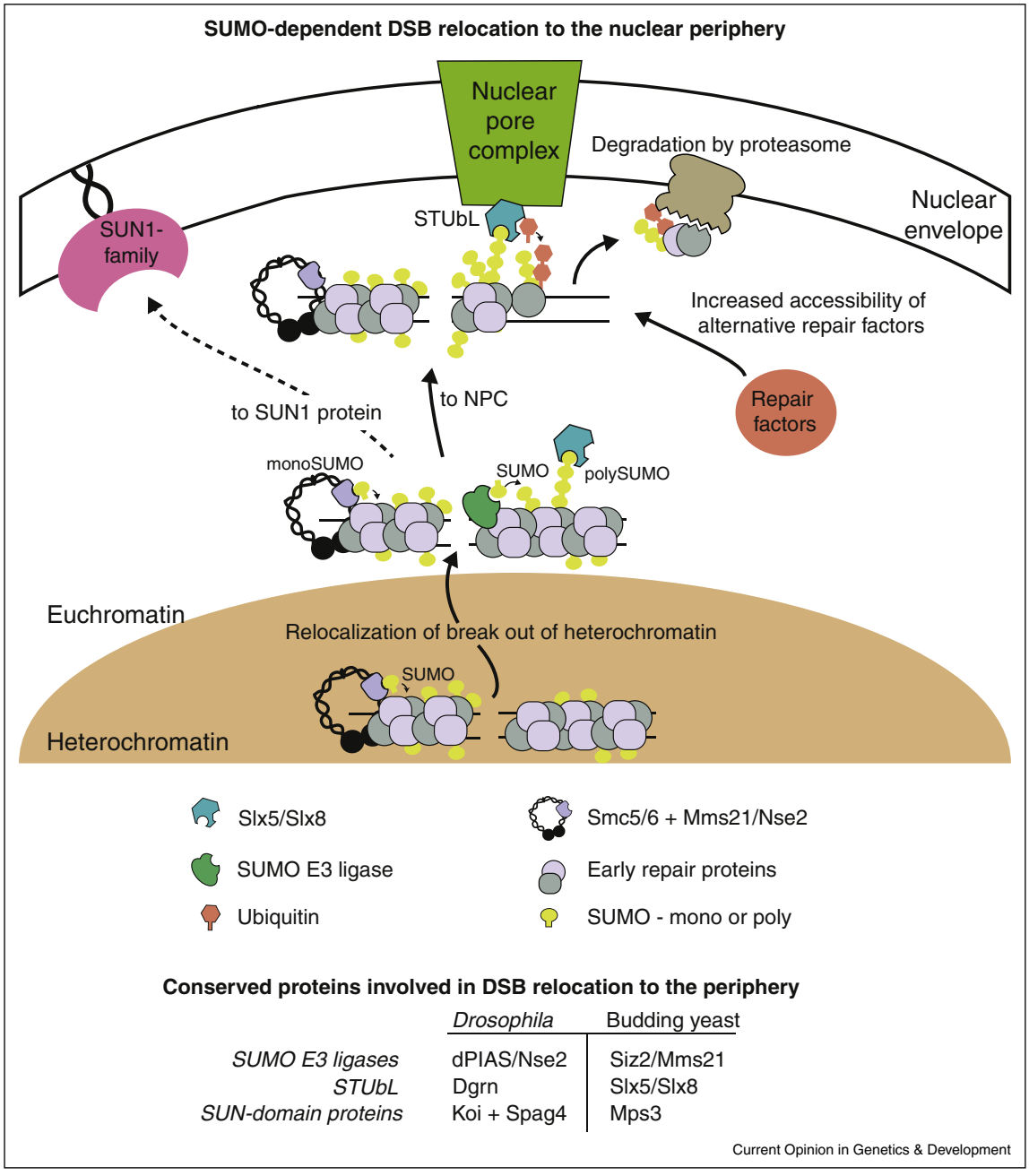
Earlier work had shown that the Slx5/Slx8 SUMO-targeted ubiquitin ligase (STUbL) not only interacts with nuclear pores, but is also recruited to persistent DSBs, both in yeast [10,46••] and in *Drosophila* [20••]. It was therefore examined whether Slx5/Slx8 (Degringolade or Dgrn in flies; RNF4 in mammals) was required for the relocation of DSBs to the periphery or if it acts only after recruitment. Considering that STUbLs contain small ubiquitin-like modifier (SUMO) interacting motifs (SIMs) [48], and that many repair proteins are SUMOylated [49], this role of Slx5/Slx8 immediately raised the question whether or not SUMO ligases were involved in DSB relocation. Four new papers [20••,44•,46••,50•] have examined the roles of Slx5/8 and SUMO ligases at DSBs, eroded telomeres and collapsed replication forks in budding yeast and *Drosophila*, producing a coherent picture of the role of SUMO and its ligands in break relocation (Figure 1).

In *S. cerevisiae*, there are four SUMO E3 ligases, Siz1, Siz2 (mammalian PIAS homologs), Mms21 (which binds the Smc5/6 complex), and the meiosis specific Cst9. SUMOylation events mediated by both Siz2 and Mms21 are implicated in DSB relocation to the nuclear periphery [46••]. Interestingly, the relocation has different requirements during the cell cycle. PolySUMOylation by Siz2 or Mms21 in G1 phase recruits Slx5/Slx8 to the break which then allows relocation. An artificial poly-SUMO construct was sufficient to shift an undamaged site to NPCs, in a Slx5-dependent manner, while a similarly targeted mono-SUMO construct was not able to [46••]. In S phase, on the other hand, monoSUMOylation was sufficient to shift resected damage to the SUN-domain protein, Mps3, in a manner independent of Slx5/Slx8 [46••]. This is reminiscent of an earlier report that a targeted yKu80-SUMO fusion shifts internal loci and/or telomeres to Mps3 [51]. Thus, there are cell cycle-, and SUMO chain-dependent pathways that direct damage to one or another perinuclear processing sites, obviously with different repair outcomes.

At pores both imprecise non-homologous end joining (NHEJ) and break-induced replication (BIR) are compromised by mutations in Nup84 (the binding site for Slx5) and by loss of the STUbL itself [46••]. This observation is bolstered by the fact that the tethering of a subtelomeric DSB to the NPC resulted in hyperactive BIR, as well as moderately increasing imprecise NHEJ [24•]. In an analogous study using *Drosophila* cells, Chiolo and colleagues first showed that DSB relocate away from heterochromatin to enable recombination to occur [19]. This required both SUMOylation by SUMO E3 ligases, and the *Drosophila* Slx5/Slx8 equivalent Dgrn [20••]. However, in flies not only the NPC, but also the Mps3 homologues, Koi and Spag4, appear to recruit the STUbL (Dgrn) and its RENi cofactor (Rad60) to the periphery. These work in concert with the Smc5/6-SUMO ligase complex (Mms21), triggering the recruitment of heterochromatic DSBs to pores [20••]. It is proposed that in yeast, the proximity of the proteasome to the NPC justifies relocation, while in flies it is unclear whether further processing of the break or protein degradation of a STUbL target, is necessary for repair.

Importantly, it is not only artificially induced breaks that find their way to the nuclear periphery: two important recent studies show that both eroded telomeres and replication damage associated with expanded triplet

**Figure 1**




**Relocation of a DSB to the nuclear periphery in yeast and Drosophila**.
DSBs can occur in heterochromatin or euchromatin. SMC5/SMC6 and its associated E3 ligase Mms21, mediate monoSUMOylation which allows DSBs to shift out of heterochromatin and enable repair. Recruitment of additional SUMO E3 ligases (e.g. Siz2/PIAS homologues) to the DSBs promotes polySUMOylation which facilitates STUbL dependent relocalization of the lesion to the NPC, where proteins are ubiquitylated and degraded by the proteasome. This is thought to allow alternative repair factors to bind the DSB, mediating BIR or imprecise NHEJ. MonoSUMOylated DSBs can also shift to SUN-domain proteins embedded in the nuclear envelope independent of STUbL interactions. This occurs particularly in S-phase cells where breaks are readily resected and bound by Rad51.

repeats, shift transiently to pores for processing and release [44•,50•]. The Lisby and Geli laboratories looked at telomeres in a telomerase-deficient yeast strain, and found that shortened telomeres are relocated to the NPC in a very similar SUMO-dependent pathway. The shift, and Slx5/Slx8 itself were both required to enable recombination-mediated elongation of the short terminal TG-tract, generating type II survivors in which TG

repeats are maintained by recombination (ALT in mammals) [50•]. Finally, an analysis of expanded CAG triplet repeats, which serve as hot spots for replication fork collapse in S phase, showed that these also relocate transiently in late S phase to the NPC, again in a Slx5/8-dependent manner [44•]. Unlike flies, the Mps3 protein was not involved. Failure to recruit the CAG repeat to the periphery led to both expansions and deletions of the CAG tract [44•]. Taken together, these studies collectively define a conserved pathway through which damage is shifted from its normal subnuclear context to the nuclear pore, in a manner dependent on SUMOylation. Failure to move appears to be detrimental to recovery (Figure 1), and the shift of damage to a favored site of repair in all cases depends on SUMOylation.

Nonetheless, many open questions remain. It remains unclear whether one or many proteins are SUMOylated, and which are degraded following STUbL-mediated ubiquitination. Epistasis mapping studies place the proteasome in the same pathway as Nup84 and Slx5/Slx8 for the recovery from difficult-to-repair breaks [10], yet it is unclear why targeted protein degradation must occur near the pore. What is gained by clustering or targeting damage through SUMOylation and SIM-containing proteins? An alternative hypothesis proposes that the nuclear periphery serves to bring free ends or common sequences together, so that the homology search for difficult-to-repair breaks becomes a 2-, rather than 3-dimensional search.

Besides the nuclear envelope, the nucleolus which harbors the rDNA repeats, is a major organizing element of the nucleus. Previous studies in *S. cerevisiae* found that DSBs induced in the rDNA context, also shift away from the nucleolus to allow break processing and Rad51 loading, and repair by homologous recombination [52]. The shift out of the nucleolus depended on the SMC5/6-Mms21 SUMO ligase, and in this case it appeared that Rad52 was the essential target of SUMOylation. Failure to modify Rad52 and shift away from the nucleolus, resulted in aberrant recombination events [52]. Two new studies have addressed this issue in mammalian cells [53•,54] with results remarkably similar to those from budding yeast. Persistent nucleolar DSBs were observed to shift from the core of the nucleolus to its periphery [53•,54]. While Haring *et al.*, found that most DSBs in the rDNA were efficiently repaired by NHEJ, both studies showed that persistent DSBs led to an ATM-dependent inhibition of Pol1 transcription, and nucleolar rearrangements. The relocation of the rDNA break from the interior of the nucleolus to its periphery allowed HR factors to be recruited [53•]. This supports the notion that certain chromatin compartments are refractory to repair, apparently in all eukaryotic organisms. Domains that are rich in repeats appear to require special measures and tailor-made pathways for DSB repair. The parallels in the roles of chromatin movement, SUMOylation, and nuclear pores in DSB repair from yeast to humans, as highlighted above and in many other recent studies [55,56,57,58], secures this as a highly promising field of research.

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